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Biology of *Prodiplosis longifila* Gagné and population fluctuation in tomato crops sprayed with insecticides

Biología de *Prodiplosis longifila* Gagné y fluctuación poblacional en cultivos de tomate asperjados con insecticidas

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Keywords:
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ABSTRACT

Laboratory studies were carried out to determine life history parameters of the bud midge *Prodiplosis longifila*, a key pest of tomato. *P. longifila* eggs collected from tomato fields hatched into larvae within 1.2 days average and development time (larva I to adult) was around 14 days at environmental conditions. Sex ratio (male:female) of *P. longifila* was 1:1.03. Longevity of both male and female *P. longifila* adults (1.1 days for both of them) increased after sugar feeding. Field trials to determine population fluctuation of *P. longifila* were conducted in three commercial tomato plots located in Colombia, under calendar-based insecticide treatments. Average numbers of live *P. longifila* larvae in all plots were higher during the last two weeks of sampling than during the first two. Larvae numbers increased even under insecticide spraying. Larvae numbers increased after flowering, suggesting that adults were attracted to tomato flowers and probably used them as source of sugar. Infestation (%) was positively correlated with average number of larvae/leaf bud, suggesting the potential of this indirect method to monitor *P. longifila* larvae in tomato. Insecticide sprays, applied to tomato fields to control *P. longifila*, on a calendar-based regime, did not reduce larvae density.

Palabras clave:
Daño
Infestación
Longevidad
Tiempo de desarrollo
Proporción de sexos
Floración

RESUMEN

Se llevaron a cabo estudios de laboratorio para determinar parámetros de historia de vida del mosquito de las agallas *Prodiplosis longifila*, una plaga clave del tomate. Huevos de *P. longifila* colectados en cultivos de tomate eclosionaron, en promedio, en 1,2 días y el tiempo de desarrollo (larva I hasta adulto) fue alrededor de 14 días a condiciones ambientales. La proporción de sexos (macho: hembra) de *P. longifila* fue de 1:1.03. La longevidad promedia del macho y de la hembra (1,1 días ambas) se incrementaron después de consumir azúcar. Ensayos de campo para determinar la fluctuación poblacional de *P. longifila* se desarrollaron en tres lotes comerciales de tomate en Colombia bajo aspersiones de insecticidas tipo calendario. Los números promedio de larvas en todos los lotes fueron mayores durante las dos últimas semanas que durante las dos primeras semanas de muestreo. El número de larvas aumentó aún bajo la aspersión de insecticidas. El número de larvas aumentó después de la floración, sugiriendo que los adultos fueron atraídos a las flores de tomate y probablemente las utilizaron como fuente de azúcar. La infestación (%) estuvo correlacionada positivamente con el número promedio de larvas/brote foliar, sugiriendo el potencial de este método indirecto para monitorear larvas de *P. longifila* en tomate. La aspersión de insecticidas en cultivos de tomate para controlar *P. longifila* en un esquema basado en el calendario no redujo la densidad de larvas.

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Horticultural production may be attractive to small farmers from developing countries, as it can reduce poverty (Weinberger and Lumpkin, 2007), increase food security (Altieri and Toledo, 2011), diversify diets and reduce micronutrient malnutrition (Tontisirin *et al.,* 2002).

Vegetable farming in developing countries is affected by pests that cause major losses at the commercial level. The farmers control these pests mainly with spraying insecticides on calendar-based regimes, to meet market quality (Wyckhuys *et al*., 2013). Pesticide traceability is an additional barrier for developing countries to compete in the international fruit and vegetable market (Weinberger and Lumpkin, 2007), as rigorous laws and regulations to control pesticide use are still lacking in these countries (Ecobichon, 2001).

In this context, tomato (*Solanum lycopersicum* L. (Solanaceae)) is one of the most widely consumed vegetables in the world. The world´s production of fresh tomato amounts 141,101,758 tones, grown on 4,250,162 ha (FAO, 2016). Tomato growing in developing countries requires intensive use of insecticides to control severe insect pests (Matthews *et al.,* 2003). In South America, tomato production is limited by several pests, including whiteflies *Bemisia tabaci* Gennadius and *Trialeurodes vaporariorum* (Westwood) (Cardona *et al.,* 2001), the fruit borer *Neoleucinodes elegantalis* Guenée (Diaz *et al.,* 2013), the tomato leaf miner *Tuta absoluta* (Meyrick) (Lietti *et al.,* 2005) and the bud midge *Prodiplosis longifila* Gagné (Diptera: Cecidomyiidae).

Podiplosis longifila has been reported to attack a number of crops in some countries of South America. In Peru it attacks more than fifteen horticultural crops (Diaz-Silva, 2011) including asparagus, tomato, potato, bean and cucurbits (Diaz-Silva, 2011; Goldsmith *et al.,* 2013; *Kroschel et al., 2012*). In Ecuador *P. longifila* also attacks several horticultural crops as tomato, potato, soybean, bean, bell pepper (Valarezo *et al.,* 2003). In both countries *P. longifila* feeds on several plants growing around main crops (Diaz-Silva, 2011; Valarezo *et al.,* 2003). In Colombia *P. longifila* has been found feeding on tomato, bell pepper and Tahiti lime (Hernandez *et al*., 2015). Being tomato the main crop damaged by *P. longifila* and because of the risks posed by importing tomatoes, *P. longifila* was recently added to the alert list of the European and Mediterranean Plant Protection Organization (EPPO, 2015). In Colombia, *P. longifila* is widespread over tomato crops and is causing important economic losses (Hernandez *et al.,* 2015). Additionally, *P. longifila* was a pest in the USA causing economic damage to the flower buds of Tahiti lime (Peña *et al.,* 1989).

P. longifila adults are difficult to see in the field during daylight hours, as they are active at dusk. Females lay their eggs on leaf buds and flowers as well as under the calyx. After egg hatching, larvae undergo three instars before becoming pupae (Gagné, 1994). Larvae suck juices from the epidermal tissues of leaf buds, flowers and small fruits (Hernandez *et al.,* 2015), resulting in damaged fruits with low commercial value (Valarezo *et al.,* 2003). *P. longifila* larvae are small and difficult to detect under field conditions therefore biological information is required to understand the development of the insect in the crop. Due to the severe damage caused by the insect to tomato crops, the first objective of this research was to study some biological parameters (*e.g.*, development time, sex ratio and adult longevity) of *P. longifila* under laboratory conditions as these parameters may explain differences in abundance and numerical changes over the crop season contributing to develop pest management strategies. Commercial tomato varieties currently in use are susceptible to *P. longifila* and resistant varieties are not yet available (Mena *et al.,* 2014). Strategies to control *P. longifila*, including light traps with sticky yellow panels (Lazarte and Tupes, 2015) and other cultural and mechanical methods (Diaz-Silva, 2011), have been proposed (Kroschel *et al.,* 2012; Goldsmith *et al*., 2013) and some natural enemies identified (Cedano and Cubas, 2012; Peña *et al.,* 1989; Diaz-Silva, 2011). However, tomato farmers mainly use chemicals to control *P. longifila* (Valarezo *et al.,* 2003), as evidenced in field surveys carried out in tomato growing areas of Colombia, during 2011 and 2012 (Hernandez, 2014). Therefore, based on the geographical expansion of *P. longifila* on tomato crops in Colombia (Hernandez *et al.,* 2015), the second objective of this research was to determine the impact of insecticide sprays on *P. longifila* populations in Colombian commercial tomato fields.

MATERIALS AND METHODS

Sampling and identification of *Prodiplosis*

Larvae of *Prodiplosis* were sampled in tomato (*Solanum*

lycopersicum L.) fields located in El Encanto (Palmira, 03°32'22.7"N; 76°21'12.7"W), Valle del Cauca, Colombia. New leaves and reproductive structures showed symptoms of *P. longifila* damage (Hernandez *et al*., 2015). Collected material was taken to the laboratory in plastic cages (20x10 x5 cm) containing wet tissue paper to avoid desiccation. Part of the larvae were used for biological studies (see below) and some of them were allowed to develop into adults to confirm the taxonomic identity of the insect. Eggs of *Prodiplosis* were collected from tomato leaf buds and transported to the laboratory in Petri dishes (5.5 mm x 10 mm) lined with water-wet filter paper. *Prodiplosis* males were prepared for microscopy mounting (Hernandez *et al.,* 2015) and identified using Gagné's, (1994) taxonomic keys.

Biology of *Prodiplosis longifila*

Eggs of *Prodiplosis* were observed daily, using a microscope (Nikon ZMZ800), until they hatched. To determine development time from larva to adult, first instar larvae of *Prodiplosis,* collected from the field, were kept on tomato leaf buds, inside air-sealed plastic bags (Valarezo *et al.,* 2003), until larvae reached the third instar, recognizable by their yellow colour and jumping activity. Third instar larvae were transferred to Petri dishes (100 x 15 mm) with wet sterilized screened soil, to allow pupae formation. Petri dishes were sealed with Plastifilm®, to collect emerging adults. Part of the adults were killed in alcohol (75%) to determine sex ratio. Remaining adults (n = 140) were kept alive to determine adult longevity by setting aside individual recently emerged adults inside microcentrifuge tubes (1.5 mL) containing a cotton ball impregnated with a sucrose solution (50% m/v) or just with water as a control. Adult survival was observed every day. Experiments were carried out at 26.2 °C ± 1.34; 61.5 % ± 6.3 RH; 12 L:12 D photoperiod.

Population fluctuation of *Prodiplosis longifila* in tomato fields

The fluctuation of *P. longifila* populations was studied in the three commercial tomato plots (planted to "Chonto" and "Cherry" S. *lycopersicum* cultivars). Plot 1 (3200 m^2) had 3800 plants of both "Chonto" and "Cherry" cultivars; plot 2 (3090 m^2) had 3678 plants of "Cherry" cultivar and plot 3 (2060 m^2) had 2452 plants of "Cherry" cultivar. Plants were planted at standard cropping distances (0.6 m x 1.4 m) and were grown under drip irrigation. Plants received weekly sprays of several insecticides, as per farmer´s decision, applied alone or as a mixture, to control tomato pests (spirotetramat against *Prodiplosis longifila* and *Bemisia tabaci*; metomil, tiametoxam and lambda-cyhalothrim against *Tuta absoluta* and *Bemisia tabaci*; dimetoato against *B. tabaci*; thiocyclam hidrogenoxalato, clorpirifos and lambda cihalotrina against *T. absoluta*). Sampling of plots 1, 2 and 3 started 103, 48 and 22 days after planting, respectively. Insect sampling on plot 3 started once the flowering and fruiting periods of plots 1 and 2 had finished. Only plot 1 had flowers and fruits when sampling started. Sampling of plots 2 and 3 started simultaneously and was carried out during six months, until the end of the crop´s reproductive period. All crop plots were sampled during the vegetative and reproductive periods. To sample *P. longifila* larvae, three crop rows were randomly selected, and 10 leaf buds were randomly sampled from each (30/ week/tomato plot). The number of leaf buds infested (with live or dead larvae) or damaged by *P. longifila* (without larvae) were counted. Leaf buds were transported to the laboratory, where the number of *P. longifila* larvae were counted. Data were used to calculate the average number of larvae per leaf bud; the percentage of larvae infestation (Equation 1) and the percentage of larvae damage (Equation 2) were calculated using Chavez´s method (Chavez, 2002). To determine the influence of chemical control on the population dynamics of *P. longifila*, dates of insecticide sprays (with spirometramat as active ingredient) to control *Prodiplosis* as well as numbers of live larva/leaf buds were recorded, comparing the initial and final sampling periods. Rainfall influence on average number of *P. longifila* larvae was studied. Data on daily rainfall at the study site was provided by the Centro de Investigación de la Caña de Azúcar Cenicaña from data collected at the local airport's meteorological station.

$$Infestation\ \% = \frac{Number\ of\ infested\ leaf\ buds}{Total\ number\ of\ leaf\ buds} x\ 100 \quad (1)$$

$$Damage\ \% = \frac{Number\ of\ damaged\ leaf\ buds}{Total\ number\ of\ leaf\ buds} x\ 100 \quad (2)$$

Statistical analysis

As the normality Kolmogorov-Smirnov and the Equal Variance tests failed (P<0.0001), differences between mean values for the immature development time were compared, using the Mann-Whitney Rank Sum Test. Biological parameters and the number of larvae/leaf bud

were expressed as average ± SE (the standard error). Sex and food influence on adult longevity was analysed, using the non-parametric Two-way Analysis of Variance General Linear Model, followed by multiple comparisons (Student-Newman-Keuls Method $P<0.05$). Field data for larva comparison were transformed to $\sqrt{X+0.5}$. To determine the effect of insecticide sprays on the survival rate of *P. longifila*, numbers of live larvae in each tomato plot were counted, comparing initial and final sampling periods, using the Mann-Whitney Rank Sum Test and *t*-Test.

RESULTS AND DISCUSSION

Identification and biology of *Prodiplosis longifila*

The morphology of adults, as described in the keys of Gagné (1994), allowed the taxonomic identification of *P. longifila* individuals. *P. longifila* eggs collected from tomato fields hatched into larvae within 1.2 ± 0.12 days (n = 18). Development time (larva I to adult) was 13.98 ± 0.35 days (n = 52). This value was lower (13.98 days) than that (17.25 days) reported by Valarezo *et al.* (2003) in tomato, probably because these authors used a lower temperature of 24 °C and development time is inversely related to temperature. To compare host plants, the development time we report in tomato is longer than the value (9 days ± 1.63) reported in Tahiti lime by Peña *et al.* (1989) at 27 °C, 84 ± 2% RH. Results show that *P. longifila* develops slower in tomato than in Tahiti lime, suggesting that it can potentially become an important pest of Tahiti lime in Colombia, where it was recently reported (Hernandez *et al.,* 2015), as occurred in the USA (Peña and Duncan, 1992).

Sex ratio (male:female) of *P. longifila* was 1:1.03. *P. longifila* adult longevity was influenced by food (Non-parametric Two-Way Anova F = 49.010, $P<0.0001$) but not by insect sex (F=1.015, $P=0.317$) or by food x insect sex interaction (F=0.403, $P=0.5274$). The sex ratio of *P. longifila* found in tomato was different from the male:female sex ratio of 1.53:1 reported by Rodriguez, (1992) in tomato and cited by Goldsmith *et al.* (2013). The sex ratio of cecidomyiids can be affected by several factors such as genetic mechanisms, differential sex mortality during diapause, mating time close to emergence (Smith *et al.,* 2004) and, sex ratio does not seem to be adjusted in response to host quality (Dorchin and Freidberg, 2004). Studies on three monogenous species (individual females that produce unisexual broods) of gall midges, a population sex ratio of 1:1 was found which was attributed to a ratio of 1:1 between female-producers and male-producers in the population (Dorchin and Freidberg, 2004). However, that study was carried out in gall forming species, which it is not the case for *P. longifila*.

Longevity of sugar-fed adult insects collected in tomato plots increased significantly, from 1.1 ± 0.05 (n=40) to 3.42 ± 0.32 (n=40) days (Student-Newman-Keuls Method $P<0.05$). Sugar intake increased longevity in *P. longifila* adults, as previously reported by Valarezo *et al.* (2003) for tomato, and by Peña *et al.* (1989) for Tahiti lime. Sugar is probably a requirement for egg maturation, as recently emerged adults feed on flowers and other nectar sources (Valarezo *et al.,* 2003). Our results also confirm that tomato flowering attracts *P. longifila* adults and stimulates oviposition, as larvae density in plots 2 and 3 increased after flowers appeared (Figure 1). The cecidomyiid *Dasineura dielsi* Rübsaamen was attracted to flowers (probably to the volatiles) of the bush *Acacia cyclops* (Kotze *et al.,* 2010) and the cecidomyiids *Megommata* sp. and *Resseliella* sp. fed on pollen of some Schisandraceae species and cocoa, even acting as pollination agents (Yuan *et al.,* 2008; Thien *et al.,* 2009). In addition to longevity, nectar and pollen probably increase *P. longifila* fecundity, as has been reported for several insects (Wäckers *et al.,* 2007). *Contarinia* spp. and other unidentified species of cecidomyiids use flowers as brooding places (Woodcock *et al.,* 2014). The difficulty to reproduce *P. longifila* in laboratory conditions, that we and other researchers have experienced, is probably due to young tomato plants being flowerless.

Population dynamics of *Prodiplosis longifila* in tomato plots

Figure 1 shows the fluctuation of *P. longifila* populations among the three tomato plots studied. Larvae of *P. longifila* were present until the end of the cropping season. The highest average number of larvae/leaf bud was 21.3 ± 2.7 for plot 1C, and the lowest was 7.6 ± 1.8 for plot 1B. An intermediate value of 13.7 ± 2.9 was found for plot 1A. In plots 1B and 1C, larvae of *P. longifila* were absent during the first seven weeks of sampling but they appeared after flowering. In plot 1B, average values of larvae/leaf bud increased from 0.5 to 2.2, two weeks before and two weeks after flowering, respectively. Average weekly rainfall in plots 1A, 1B and 1C was 0.705, 1.240 and 2.954 mm, respectively.

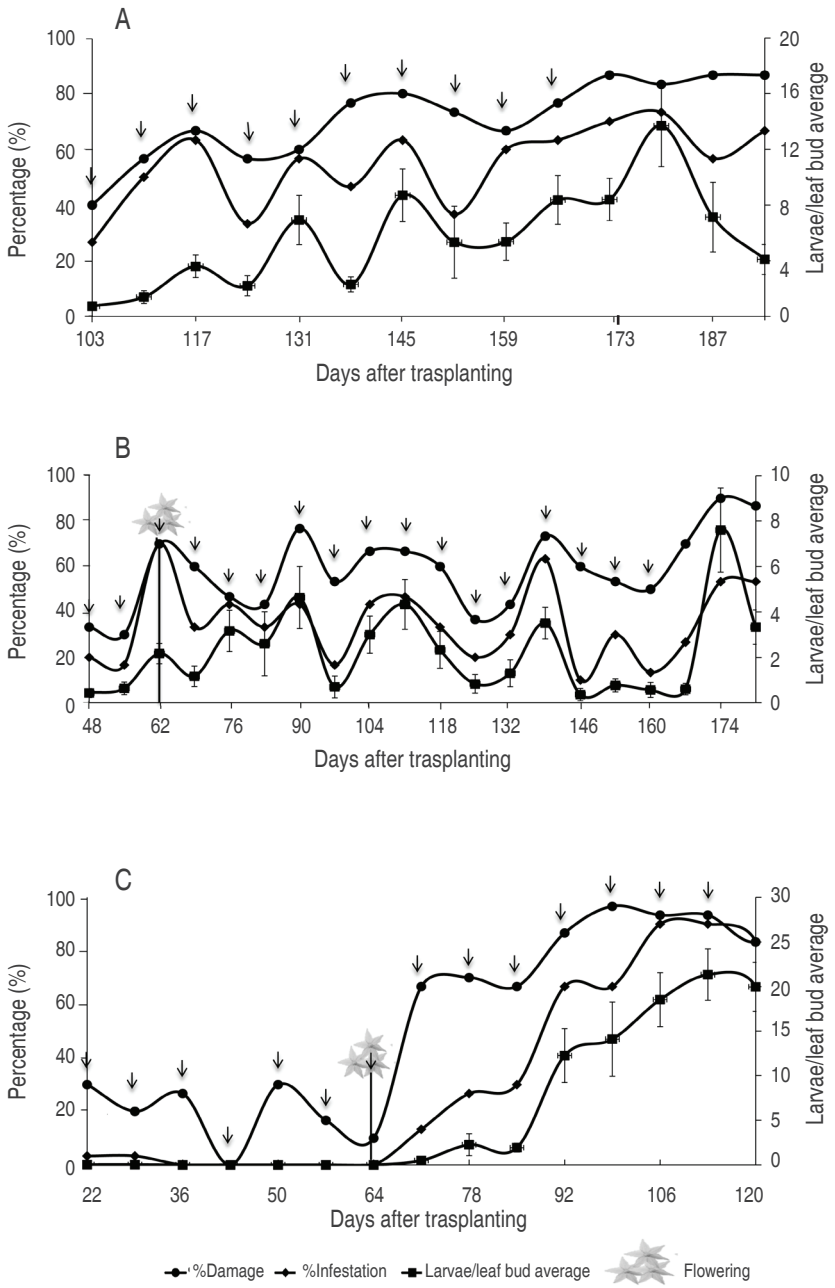

Figure 1. Fluctuation of populations of *P. longifila* in tomato plots sprayed with insecticides; days after planting, expressed as damage (%), infestation (%) and average numbers of larvae/leaf bud (mean ± SD). A. Chonto & Cherry tomato plot 1, B. Cherry tomato plot 2, C. Cherry tomato plot 3.

Correlation values between the different measured variables are presented in Table 1.

Results on the fluctuation of *P. longifila* larvae populations showed a positive correlation between damage (%) and infestation (%), and between average numbers of larvae/leaf bud and damage (%), which suggests that damage (%) could be the basis to develop an indirect method to monitor the presence of *P. longifila* in tomato fields. There is some concern, however, that the damage to leaflets on plot 1,

where *P. longifila* sampling started late in the season (103 DAT), may be old, which could probably account for the low correlation between damage (%) and infestation (%) on plot 1 (Pearson value = 0.673). Concerning climatic conditions, rainfall is a variable associated with the presence/absence of *P. longifila* in Colombia, as it could produce larva drop-off from leaves (Hernandez *et al.,* 2013) and reduction of *P. longifila* populations (Valarezo *et al.,* 2003). This effect, however, was not found in this study, probably because insecticide sprays masked its influence.

Table 1. Correlation between the different variables measured for *Prodiplosis longifila* larvae in tomato plots sprayed with insecticides.

	Damage (%) vs. Infestation (%)
Plot 1A*	Pearson = 0.7255, *P*=0.00331, n=14
Plot 1B*	Pearson = 0.6731, *P*=0.00114, n=20
Plot 1C*	Pearson = 0.9106, *P*=0.00000240, n=15
	Infestation (%) vs. Average number of larvae/leaf bud
Plot 1A*	Pearson = 0.7199, *P*=0.0369, n=14
Plot 1B*	Pearson = 0.7125, *P*=0.000423, n=20
Plot 1C*	Pearson = 0.79613, *P*=0.000000011, n=15
	Rainfall (mm) vs. Average number of larvae/leaf bud
Plot 1A	Pearson = - 0.0744, *P*=0.97985, n=14
Plot 1B	Pearson = - 0.342, *P*=0.140, n=20
Plot 1C	Pearson = -0.0985, *P*=0.7269, n=15
	Days after planting vs. Average number of larvae/leaf bud
Plot 1A*	Pearson = 0.359733, *P*=0.000972, n=120
Plot 1B*	Pearson = 0.3897838 *P*=0.0000108, n=120
Plot 1C*	Pearson = 0.4399 *P*=0.000000495, n=120

* Statistically significant correlation

In all plots, average numbers of live larvae (n=60 leaf bud samples/plot) were higher at the last two weeks of sampling than at the first two weeks: 2.25 ± 0.1976 vs. 1.10 ± 0.0789 for plot 1 (Mann-Whitney Rank Sum Test T = 2866.5, *P*<0.0001), 1.975 ± 0.1871 vs. 0.906 ± 0.0599 for plot 2 (*t* = -5.44, 118 df, *P*<0.0001) and 2.587 ± 0.2814 vs. 0.724 ± 0.0121 (Mann-Whitney Rank Sum Test T = 2791.0, *P*<0.0001). Insecticide sprays did not reduce the number of larvae, even if sprays were maintained until the end of the sampling period, as in plot 1C (Figure 1).

As expected, calendar-based insecticide spray applications, carried out even when larvae density was low, did not reduce larvae number. Paradoxically, larvae increased after sprays. Even in plot 1A, where sprays stopped 165 days after transplanting (DAT), 12 larva/leaf bud were recorded at the end of the cropping season (on day 190), a value close to the average number (13.7) recorded during the whole cropping season, suggesting the practically null effect insecticides had on larvae density. Likewise, in plot 1C, the average number of larvae/leaf bud increased from zero (64 DAT) to 20 (120 DAT), even after 8 applications of spirometramat.

Our results show that calendar-based insecticide sprays do not hamper the development of *P. longifila* larvae

populations. On the contrary, the insects remained in the area and dispersed to new plots. The average numbers (7.6, 13.7 and 21.3 larvae/leaf bud) of *P. longifila* larvae found were higher than the average values (7.2 and 0.15 larvae/leaf bud) reported by Chavez, (2002) and Mena *et al.* (2014), respectively, for unsprayed tomato plots. Chavez, (2002) used the tomato hybrids "Bingo" and "Heatwave" and Mena *et al.* (2014) tested "Unapal-Maravilla", a tomato cultivar susceptible to *P. longifila*. Because commercial tomato cultivars resistant to *P. longifila* are not yet available, as resistance traits are still being explored on wild tomato accessions (Mena *et al.,* 2012), tomato cultivars used in our experiments may partially explain the variation of larvae population density of *P. longifila*.

Spirometramat was expected to reduce larvae density, because of its plant systemic activity (Brück *et al.,* 2009), but as this did not occur, the resistance of *P. longifila* to spirometramat should be studied, considering that resistance of tomato pests, such as whiteflies and *Tuta absoluta*, to insecticides has been documented.

It is remarkable that the parasitoids *Synopeas* spp. (Hymenoptera: Platygasteridae) were not recovered from any larvae of *P. longifila* during the field study, as occurred in an unsprayed experimental tomato plot set at the same geographical area (Hernandez, 2014), probably due to insecticide spraying. Spirometramat, however, has been reported to be compatible with some parasitoid species (Garcerá *et al.,* 2013; Vanaclocha *et al.,* 2013); although it reduced the longevity of *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae; Moens *et al*., 2012), and farmers also sprayed other pesticides during our study. Species of *Synopeas* parasitize *P. longifila* feeding on different crops (Peña *et al.,* 1989; Valarezo *et al.,* 2003; Diaz-Silva, 2011).

CONCLUSIONS

Biological information obtained about *P. longifila* in tomato revealed that eggs hatched into larvae within 1.2 days and development time (larva I to adult) was around 14 days at laboratory conditions. Sugar feeding increased the longevity of *P. longifila* adults. Our results indicated that spraying spirometramat on tomato fields did not control *P. longifila* populations. *P. longifila* resistance to spirometramat should also be further studied. It is, therefore, urgent to develop and implement an integrated pest management (IPM) program for this pest. Infestation (%) was positively correlated with average number of larvae/leaf bud, suggesting the potential of this indirect method to monitor *P. longifila* larvae in tomato. Management practices should be intensified after tomato flowering when adults are attracted to the crop, *P. longifila* larva populations increase and in around two weeks a new generation of adults will be developed.

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